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LOCAL SERIAL CORRELATION IN BEHAVIORAL
STATES IN THE MOUSE

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LOCAL SERIAL CORRELATION IN BEHAVIORAL STATES IN THE MOUSE

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ABSTRACT

In the mouse, Mus musculus, local (ultradian) serial correlation (LSC) within the sleep and wakefulness states varies significantly as a function of circadian phase. The amplitude of the correlation function is greatest during the active phase of the mouse's diurnal cycle. Additionally, mean LSC (averaged across circadian phase) is significantly positive and monotonically decreasing for a broad range of short lags (1 - 23 minutes). Thus there is no evidence of positive, periodically occurring peaks in the correlation function separated by patches of small or negative correlations that is characteristic of a rhythmic process of fixed period. These findings challenge the concept that an ultradian oscillator plays a significant role in the temporal control of sleep-wake state.

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The regularly sampled, sleep-wake behavior of a mouse can be described stochastically as a time series in which a binary valued random variable fluctuates between the two possible states. It is readily appreciated that such a series is not entirely random, but instead exhibits significant local serial correlation (LSC) across a range of time intervals (lags). This correlation results in part from the organization of the alternating episodes of the two states into consolidated "bouts" of sleep and wake (1). Serial correlation beyond the mean length of the consolidated bouts, however, would be of significant interest and could arise in one of two ways; i) as the result of a rhythmic process governing the regular recurrence of bouts, or ii) as the result of an arrhythmic process in which the lengths of successive bouts are serially correlated with one another and/or have skewed distributions. Theoretically, the two processes should be distinguishable on the basis of their resultant serial correlation functions. Practically, recognition of the differences in correlation requires time series of sufficient length and resolution.

The majority of work examining temporal distribution in mammalian behavioral state data has embraced the hypothesis that a short-period "ultradian" rhythm was the source of observed LSC. In their original characterization of the REM and NREM sleep states in human subjects, Dement and Kleitman observed a REM/NREM alternation with a period of approximately 90 minutes (2). The regularity of the REM/NREM alternation and observations of apparent local periodicity in other

psychological and physiological variables led Kleitman to postulate the existence of a unifying ultradian oscillator, a "Basic Rest-Activity Cycle" (BRAC), which transcended the sleep/wake boundary and influenced the expression of a variety of behaviors in both states (3). This attractive hypothesis led to a number of attempts to demonstrate ultradian rhythms in physiology. Most of these attempts, however, have been complicated by difficulties in (i) defining variables that can be reliably measured across the entire circadian cycle and that demonstrate clear ultradian rhythmicity, (ii) obtaining data series of sufficient length and resolution to allow definitive statement about the presence or absence of periodicity, and (iii) allowing for serial correlation arising from other sources in the analysis of time series data and, especially, in the interpretation of spectral and autocorrelation results (4).

In this paper, we report data obtained from study of the temporal sleep-wake distribution of the mouse. Three factors make our experimental system particularly well suited to study of the temporal organization of sleep-wake state and the possible contribution of a BRAC. First, we are currently capable of collecting continuous measurements of sleep-wake state sufficient in length (up to 280 days) and resolution (sampled every 10 seconds) to allow rigorous determination of the significance and consistency of apparent ultradian and circadian rhythmicity. Second, the alternation of sleep and wakefulness has obvious intuitive validity as a physiological marker of the rodent BRAC,

should one exist. Finally, though the marginal (first-order) probabilities of sleep and wakefulness in the mouse demonstrate clear circadian periodicity, both states are distributed throughout the entire circadian cycle; i.e. the probability $P(\phi)$ of sleep varies with phase, ϕ , but almost never becomes one or zero. Thus, if a BRAC continues throughout the circadian cycle, its effect on the expression of sleep and wakefulness and, in particular, on the correlation structure of those variables should be measurable at all phases of the mouse's circadian cycle. This fortuitous feature of mouse sleep data obviates the use of questionable pairings of parameters from wakefulness and sleep on the assumption that they represent multiple indicators of the same rhythmic process (e.g. REM sleep and daydreaming fantasy (5)).

Nine male mice (Mus musculus) were studied while individually housed in light-tight, insulated enclosures illuminated by externally controlled, water-jacketed fluorescent lamps. For this study, data were collected under two lighting conditions; either continuous darkness ("DD"), or 12 hours of light followed by 12 hours of darkness ("LD12:12"). Food and water were available ad libitum. EEG recording techniques and automated state-scoring methods were performed as described elsewhere (6). State was determined every 10 seconds as either active wake (wheel running), quiet wake, REM sleep or NREM sleep. (For the study reported here, active and quiet wake were pooled to form "total wake", while REM and NREM sleep were pooled as "total sleep.") We selected fourteen sections from the full-length

MEAN CORRELATION vs. LAG

WAKE

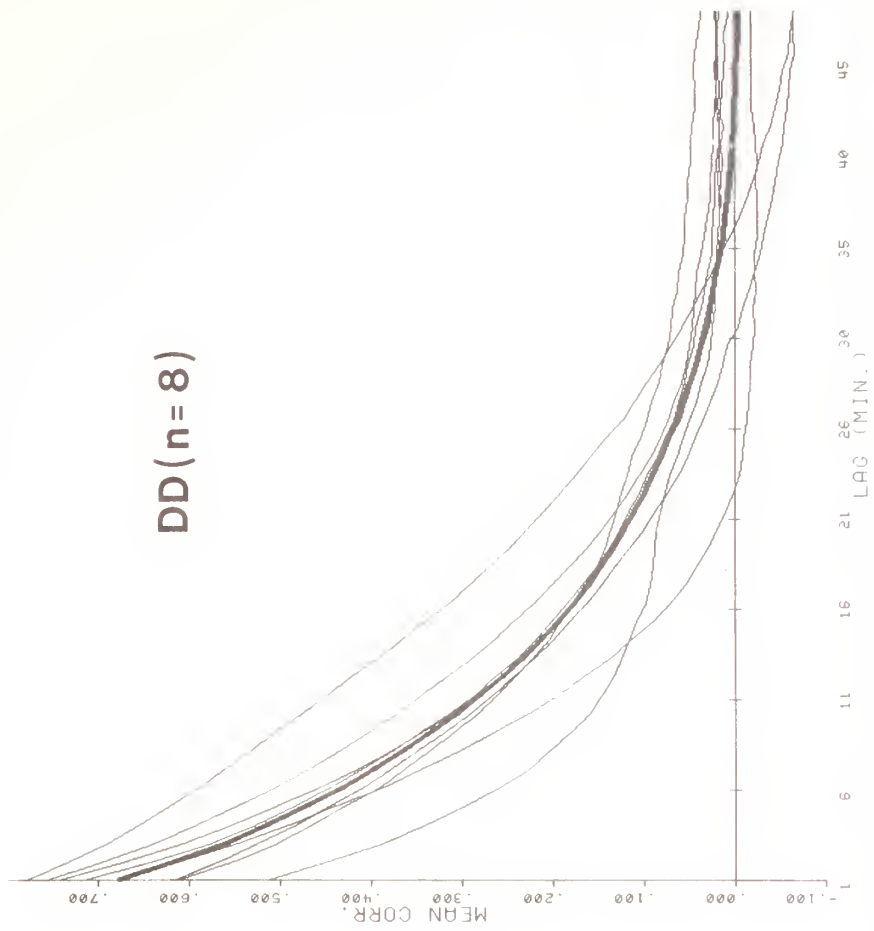
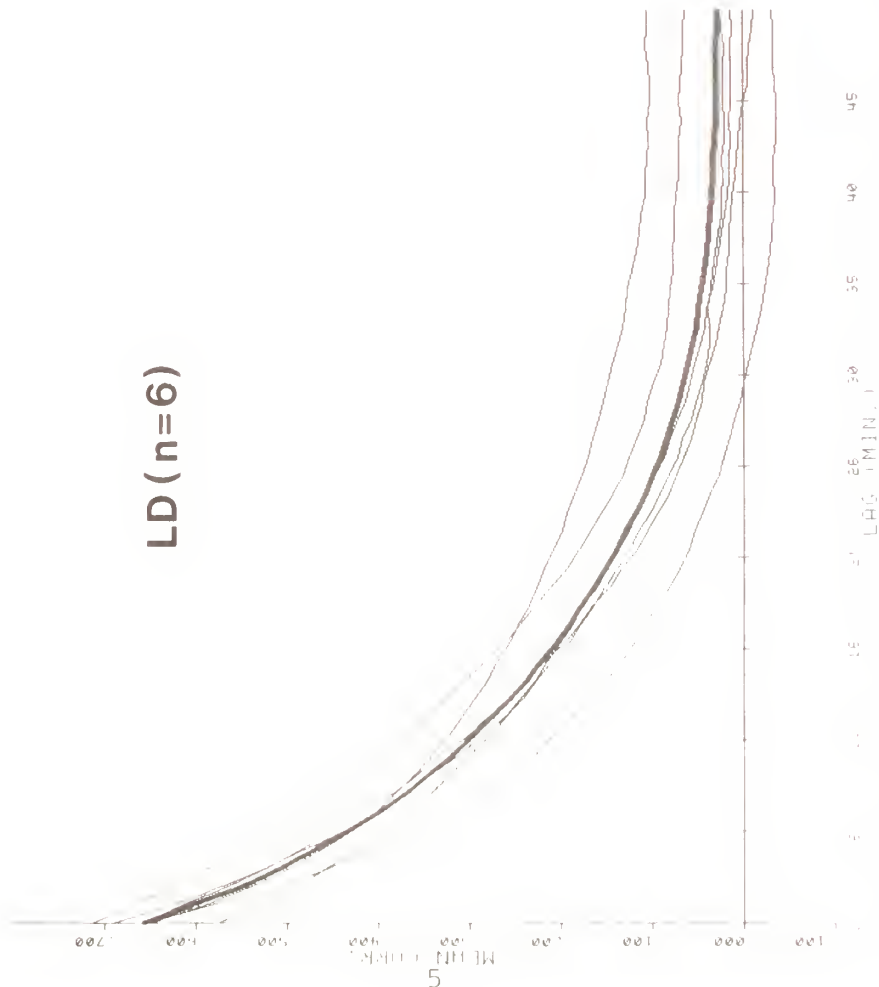


Figure 2. Estimated local serial correlation (LSC) for wakefulness, averaged across all phases of circadian cycle and plotted as a function of lag. Each individual sample (narrow lines) is plotted with the collective mean (heavy line). Panel A presents the curves for LD12:12 sections while panel B contains the DD sections. Though it is possible that correlation differs with lighting condition, no such effect is apparent from the figures.

data trains for inclusion in the analysis on the basis of (i) minimal interruption and lost data, (ii) relative stationarity of dominant circadian period (for DD samples), and (iii) total length between 20 and 30 complete circadian cycles. Eight samples were selected from the DD condition, six from LD12:12 (four mice were recorded in both experimental conditions). The data within each section were then "folded" to align the cycles along circadian phase (LD12:12 sections were aligned with clock time, DD sections were aligned using the onset of wheel-running activity as the reference phase). Data were averaged across cycles and the average was plotted as an estimate of probability of total wake or total sleep as a function of circadian phase (7). Local serial correlation (normalized joint second-order probability) was estimated across cycles at each circadian phase (8). This treatment was repeated at all lags in the range 0 - 500 minutes at steps of one minute.

The graphs in Figure 1 depict mean LSC (averaged across all phases of the circadian cycle) for each 20-30 day sample plotted as a function of lag. Note that LSC, averaged over the six LD12:12 and eight DD sections, is significantly positive (i.e. greater than 0.10) for all lags out to approximately 23 minutes. Most important, it is also apparent from Figure 1 that mean LSC is a monotonically decreasing function of lag; there are no repeated peaks and troughs either in the mean correlation curves or in any individual correlation curve that might reflect the contribution of a rhythmic process having a stable period in this range.

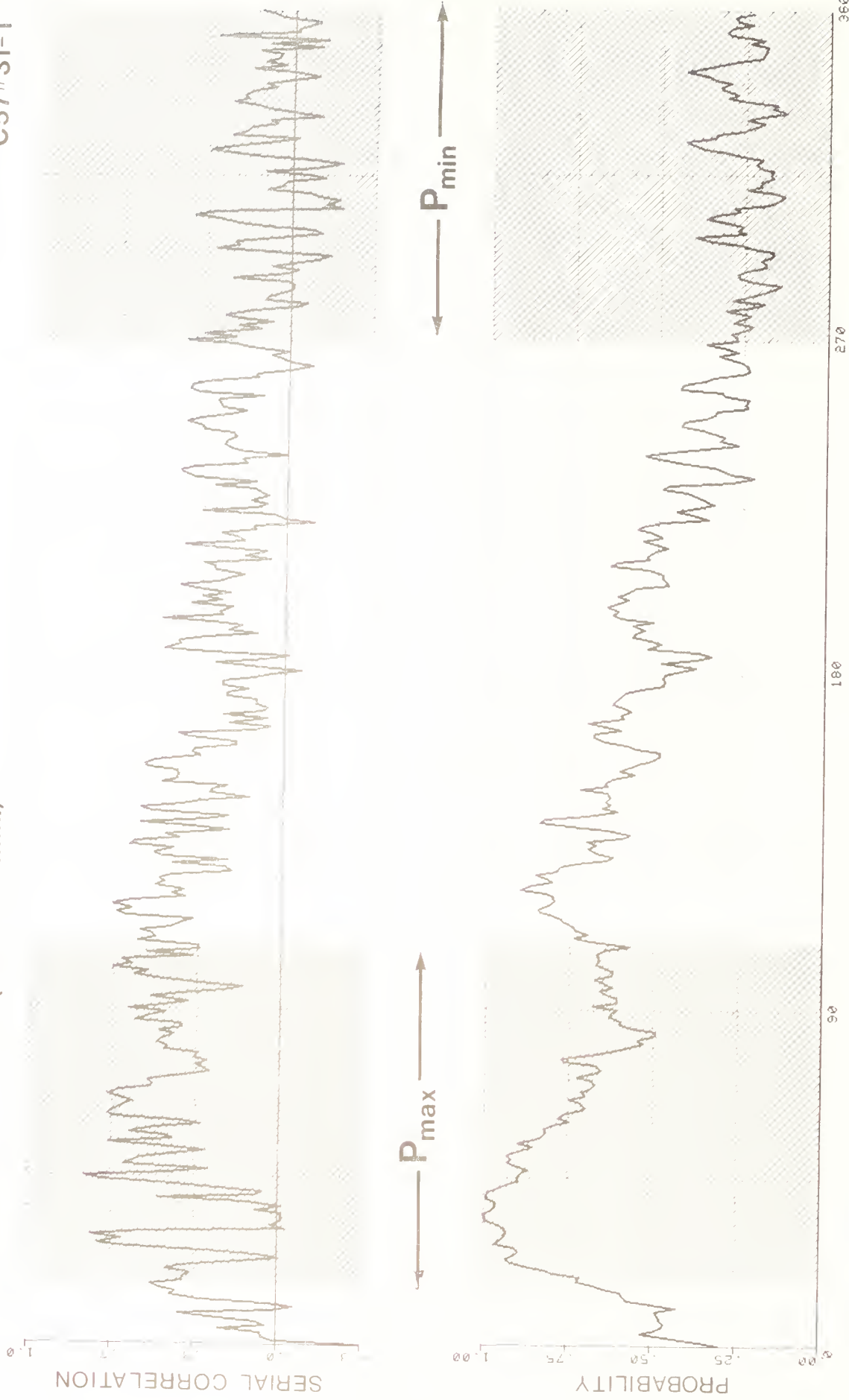
For purposes of simplification, only the representative correlational results obtained at a lag of 12 minutes were used to illustrate the subsequent analysis of phase-dependence of LSC (Fig. 2). Figure 2 presents the LSC functions at 12 minute lag for wakefulness from a representative DD section. The probability function (marginal probability of wakefulness) is plotted below the correlation function. Note that the probability of sleep should be equal to one minus the probability of wake and the correlation functions of the individual halves of such a binary process should be identical (9). In actuality, small imperfections in the state scoring process (occasional unscored or missing data) produce some deviation from the expected results. However, these differences are sufficiently insignificant to allow presentation of the data for wake as representative of the entire process.

The figure demonstrates that, although mean LSC is significantly positive at the 12 minute lag, the value of the LSC function is not consistent across the entire circadian cycle. Instead, serial correlation is maximal at phases of the circadian cycle corresponding to maximal probability of wakefulness. This specific phase dependence of the local correlation structure is a consistent feature for all mice observed, though the relationship is stronger for sections recorded in DD than for those in LD12:12 (Table 1), (10). Additionally, we found the relationship of correlation and phase to be independent of lag for a wide range of lags in the ultradian range (10 seconds to 20 minutes). (As mentioned, mean LSC approaches zero beyond lags of 20-25 minutes

WAKE

DD (tau = 1416 min.)

C57#31-1



PHASE OF CIRCADIAN CYCLE

Figure 2. Estimated ISC and estimated probability $P(\phi_0)$ for wakefulness as a function of circadian phase. The data are taken from a representative sample (Mouse #31-1) recorded for 30 consecutive circadian cycles in continuous darkness (DD). The abscissa for both plots is degrees of circadian phase (relative to activity onset) while the ordinates are estimated normalized (Pearson product moment) correlation coefficients for the upper plot and estimated probability for the lower plot. The shaded areas delimit the 90 degree windows containing the maximum and minimum mean probability values and the corresponding regions of the correlation curve. The mean values for the correlation function computed within these windows for each section were used to generate the lower plot.

TABLE 1

Local serial correlation (LSC) of wakefulness at circadian maxima and minima. Each entry represents the average of the 90 LSC values in the 90 degree phase windows encompassing the maximum and minimum probabilities of wakefulness. A t-test was used to evaluate the difference between mean correlation in the two phase windows.

Mouse and Condition	MAX PHASE	P (WAKE) LSC	MIN PHASE	P (WAKE) LSC	Δ LSC	t-test
LD01	14°	.656	264°	.160	.496	$\bar{X}/S_{\bar{X}} = 3.556$ \bar{X} <p>(p \approx .02)</p>
LD02	2°	.240	256°	.164	.076	
LD03	11°	.417	263°	.189	.228	
LD04	5°	.434	212°	.180	.254	
LD05	5°	.298	226°	.224	.074	
LD06	9°	.338	245°	.121	.217	
					$\sum X = 1.345$	
					$\bar{X} = .224$	
					$S_{\bar{X}} = .155$	
					$S_{\bar{X}} = .063$	
					\bar{X}	
DD01	7°	.626	241°	.287	.339	$\bar{X}/S_{\bar{X}} = 5.302$ \bar{X} <p>(p > .01)</p>
DD02	6°	.242	266°	.111	.131	
DD03	2°	.453	252°	.060	.393	
DD04	7°	.398	270°	.253	.145	
DD05	2°	.355	252°	.124	.231	
DD06	16°	.418	270°	.071	.347	
DD07	2°	.299	270°	.162	.137	
DD08	13°	.176	260°	.078	.098	
					$\sum X = 1.821$	
					$\bar{X} = .228$	
					$S_{\bar{X}} = .115$	
					$S_{\bar{X}} = .043$	
					\bar{X}	

and significant correlation no longer exists at any phase of the circadian cycle.)

The consistency of positive correlation across lags and the absence of significant periodic peaks and troughs in mean LSC argue against a stable ultradian rhythm as the principal source of local correlation. Further, at no lag in this 10 second to 20 minute range was local serial correlation found to be significantly negative for any circadian phase. Thus it is unlikely that the observed circadian changes in correlation are the result of either imprecise estimation of the ultradian period or an ultradian oscillator whose period changes stably with circadian phase. Although these findings alone do not preclude correlation arising from an ultradian oscillator, they do suggest that such a contribution is minor relative to the significant, general local serial correlation that was observed. To account for all observed features of the correlation structure, the variability of the ultradian "oscillator" would be so large as to defy the definition of the term.

A more plausible explanation for the observed correlation structure is that the phase of the circadian cycle corresponding to peak wakefulness is associated with a simultaneous increase in mean bout length for both states and/or an increase in the serial correlation of the lengths of successive bouts of a given state. That is, the mechanisms controlling the expression of sleep and wakefulness might be altered at this phase of the circadian cycle such that the lengths of successive bouts of a given state are correlated to a greater degree than at other

circadian phases (11). Such a model could account for both the dependence of serial correlation on circadian phase, and the occurrence of significant serial correlation at all lags in this range. Further, serial correlation arising from such a source can result in the appearance of rhythmicity in frequency spectra or autocorrelations (12), a phenomenon that may account for a number of reports of ultradian rhythmicity in physiology.

Lastly, it is clear that the absence of evidence for ultradian rhythmicity in sleep and wakefulness does not completely preclude such periodicity in the REM and NREM states. However preliminary results using the same analysis techniques presented here show no evidence of ultradian periodicity in REM and NREM states in the mouse (13). To remain viable, therefore, it would now seem that models proposing ultradian periodicity must either abandon the extension to a rest-activity cycle as proposed by Kleitman or invoke a phylogenetic origin for the rhythm after the evolution of the mouse. Given other similarities of mammalian sleep-wake organization, this latter assumption is difficult.

REFERENCES

1. The length of consolidated bouts of sleep and wakefulness varies from species to species. For the mouse, bouts are relatively short and many alternations between sleep and wakefulness occur within each daily cycle. Conversely, human sleep is organized into a few large consolidated bouts and alternations between the states are much less frequent. (H. Van Twyver, Physiol. Behav. 4, 901, 1969; H. Zepelin and A. Rechtschaffen, Brain Behav. Evol. 10, 425, (1974).
2. REM and NREM are the two basic states common to all mammalian sleep. (W.C. Dement and N. Kleitman, Electroenceph. Clin. Neurophysiol. 9, 673, 1957).
3. N. Kleitman, Sleep and Wakefulness, (University of Chicago Press, Chicago, 1963).
4. As an example Klein and Armitage (R. Klein and R. Armitage, Science, 204, 1979) purported to find an ultradian rhythm in scores of visual and verbal tests performed by human subjects performed over a whole day. A more detailed analysis (Andresen, T., "Discussion and re-analysis of experimental data in the investigation of ultradian rhythms in humans," Naval Postgraduate School Thesis, Monterey, Cal., March 1981) showed only a lunch-time effect.
5. D. F. Kripke and D. Sonnenschein, in The Stream of Consciousness, K. S. Pope and J. S. Singer, Eds. (Plenum Press, New York, 1978).

6. Electrocortigram is recorded using standard implant techniques for the mouse [M. M. Mitler and R. Levine, Psychophysiology 7, 122, 1970] and preprocessed (amplified and filtered) using a Grass Model 6 polygraph. Automated sleep state analysis is performed using software developed in our laboratory [F. A. Vincent, SQEEK - A computer algorithm for the real time automatic sleep state scoring of mouse electroencephalogram, Ph.D. Dissertation, Stanford University, 1977] and running on a PDP 11/34 computer (Digital Equipment Corp., Maynard, MA).
7. The data are treated as a binary time series X_i , $i = 0, 1, \dots$, where $X_i = 1$ if the animal is in the particular state (e.g. wake) at that point and $X_i = 0$ if he is not. The analysis is then performed by first arranging X_i in a two-way layout, modulo L , where $L =$ the estimated cycle length τ (tau) of the circadian rhythm (for animals in LD12:12, $\tau = T$, the environmental period of 24 hours). Thus $X_{(k, \ell)} = X_{(kL + \ell)}$ for (phase) $\ell = 0, 1, \dots (L-1)$ and $k = 0, 1, 2, \dots$. As the data are assumed to be stationary at any phase or phases in the circadian rhythm within the sample (consisting of m cycles of period L), the probability function, $P(\phi_\ell)$, can be estimated as

$$\hat{P}(\phi_\ell) = \sum_{k=0}^{m-1} X_{(k, \ell)} / m .$$

8. The local serial correlation is estimated as

$$\hat{r}(\ell, j) = \hat{P}(\phi_\ell, \phi_{\ell+j}) - \hat{P}(\phi_\ell) \hat{P}(\phi_{\ell+j}) / \left\{ \hat{P}(\phi_\ell) [1 - \hat{P}(\phi_\ell)] \hat{P}(\phi_{\ell+j}) [1 - \hat{P}(\phi_{\ell+j})] \right\}^{1/2}$$

where

$$\hat{P}(\phi_\ell, \phi_{\ell+j}) = \sum_{k=0}^{m-1} X_{(k, \ell)} X_{(k, \ell+j)} / m$$

is an estimate of the joint probability function.

9. For a binary time series, if \hat{P}_1 and $X_{(k, \ell)} = 1$ correspond to state 1 and \hat{P}_0 and $X_{(k, \ell)} = 0$ correspond to state 0, then we can compute $\hat{P}_1(\phi_\ell)$, $\hat{P}(\phi_\ell, \phi_{\ell+j})$ and $\hat{r}_1(\ell, j)$ as in 7 and 8 previously.

It follows that:

$$a) \quad \hat{P}_0(\phi_\ell) = \sum_{k=0}^{m-1} (1 - X_{(k, \ell)}) / m = 1 - \hat{P}_1(\phi_\ell);$$

$$\begin{aligned} b) \quad \hat{P}_0(\phi_\ell, \phi_{\ell+j}) &= \sum_{k=0}^{m-1} (1 - X_{(k, \ell)}) (1 - X_{(k, \ell+j)}) / m \\ &= 1 - \left[\sum_{k=0}^{m-1} X_{(k, \ell)} / m \right] - \left[\sum_{k=0}^{m-1} X_{(k, \ell+j)} / m \right] \\ &\quad + \left[\sum_{k=0}^{m-1} X_{(k, \ell)} X_{(k, \ell+j)} / m \right] \\ &= 1 - \hat{P}_1(\phi_\ell) - \hat{P}_1(\phi_{\ell+j}) + (\hat{P}_1(\phi_\ell, \phi_{\ell+j})) \end{aligned}$$

$$\begin{aligned}
c) \quad \hat{r}_0(\ell, j) &= \frac{\hat{P}_0(\phi_\ell, \phi_{\ell+j}) - \hat{P}_0(\phi_\ell) \hat{P}_0(\phi_{\ell+j})}{\left[\hat{P}_0(\phi_\ell) (1 - \hat{P}_0(\phi_\ell)) \hat{P}_0(\phi_{\ell+j}) (1 - \hat{P}_0(\phi_{\ell+j})) \right]^{1/2}} \\
&= \frac{\left[(1 - \hat{P}_1(\phi_\ell) - \hat{P}_1(\phi_{\ell+j}) + \hat{P}_1(\phi_\ell, \phi_{\ell+j}) \right. \\
&\quad \left. - (1 - \hat{P}_1(\phi_\ell)) (1 - \hat{P}_1(\phi_{\ell+j})) \right]}{\left[(1 - \hat{P}_1(\phi_\ell)) (\hat{P}_1(\phi_\ell) (1 - \hat{P}_1(\phi_{\ell+j})) \hat{P}_1(\phi_{\ell+j})) \right]^{1/2}} \\
&= \frac{\hat{P}_1(\phi_\ell, \phi_{\ell+j}) - \hat{P}_1(\phi_\ell) \hat{P}_1(\phi_{\ell+j})}{\left[\hat{P}_1(\phi_\ell) (1 - \hat{P}_1(\phi_\ell)) \hat{P}_1(\phi_{\ell+j}) (1 - \hat{P}_1(\phi_{\ell+j})) \right]^{1/2}} \\
&= \hat{r}_1(\ell, j) .
\end{aligned}$$

10. The apparent weakening of phase-dependence of correlation observed in LD12:12 sections is not thought to represent a true effect of lighting condition. Rather it is believed to be the result of reduced variation in the phase alignment of these sections resulting in a larger portion of the cycle in which the marginal probability of wake is near or equal to one ($P(\text{sleep}) = 0$). The correlation behaves erratically when P is very near to one and this effect results in a reduced average correlation across the 90 degree window encompassing maximum probability of wake. For DD sections, the increased phase variability of the free running rhythm results in fewer points where the marginal probability is very close to one and consequently the correlation function is better behaved.

11. Conclusions about the phase-dependence of bout length hinge on the operative definition of the bout itself. With the 10-second resolution of state measurement used in this study, single 10 second epochs often interrupt bouts of 50-120 minutes in length. Whether this conservative definition represents a reasonable interpretation of underlying biological processes is open to question.
12. Serial correlation in time series can give rise to the same effects in spectra estimated from a finite sample as those obtained from a time series consisting of a periodic signal plus noise. This result gave rise to the hunt for periodicities in economic time series, a hunt which has proved quite futile. See, for example, Granger and Hughes' re-examination of the Beveridge time-series of annual wheat prices (J. Roy. Stat. Soc. A134:413-428, 1971). See also J. T. Enright, J. Theoret. Biol., "The search for rythmicity in biological time series", 8, 426-468, 1965.
13. Richardson, G. S., Orav, E. J., Lewis, P. A. W., & Dement, W. C. (In preparation).
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